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Salvage therapy using FLT3 inhibitors may improve long-term outcome of relapsed or refractory AML in patients with *FLT3*-ITD

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Abstract

To determine the long-term efficacy of FLT3 inhibitors (FLT3i) in the salvage setting for relapsed and refractory (rel/ref) acute myeloid leukemia (AML) with *FLT3* internal tandem duplication (AML *FLT3*-ITD), we conducted a retrospective study of 120 patients with rel/ref AML *FLT3*-ITD who received salvage therapy with either FLT3i-containing regimen (FLT3i group, N = 45) or conventional cytotoxic regimen (conventional group, N = 75). The median overall survival (OS) after the first salvage in the FLT3i group was 6.9 vs. 4.6 months in the conventional group (P = 0.17). The OS was better in the FLT3i group among patients with initial complete remission (CR) duration ≥ 12 months or with primary refractory disease (6.9 vs. 3.7 months; P < 0.01). The OS was better when FLT3i was combined with cytotoxic agents versus monotherapy (17 vs. 4.8 months; P = 0.017). Multivariate analysis revealed that the use of FLT3i was an independent predictor of OS (hazard ratio 0.58; 95% confidence interval, 0.38–0.88). Incorporating FLT3i into salvage strategies may improve long-term outcome of patients with AML *FLT3*-ITD. Prospective studies to validate this conclusion are warranted.

Keywords

AML; FLT3 inhibitors; *FLT3*-ITD

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Authorship Contributions JC designed the study, conducted the research, analysed the data, and wrote and approved the manuscript; KT designed the study, conducted the research, collected and analysed the data and wrote the manuscript; HK, NP, MA, GB, SF, GGM, ZE and FR conducted the research and reviewed and approved the manuscript; SP and MCT retrieved and collected the data, conducted the statistical analysis and critically reviewed, and approved the manuscript; RL conducted the molecular testing and reviewed and approved the manuscript.

Introduction

The FMS-like tyrosine kinase (FLT3) is a transmembrane tyrosine kinase that belongs to the class III family of receptor tyrosine kinases. Internal tandem duplications (ITD) in the juxtamembrane domain of *FLT3* are found in approximately 15–30% of acute myeloid leukemia (AML) cases and constitutively activate its kinase cascades, contributing to leukemogenesis (Stirewalt and Radich 2003). The prognosis of patients with AML and *FLT3*-ITD (AML *FLT3*-ITD) is known to be significantly worse than that of with wild-type *FLT3* (*FLT3* WT), largely a result of a higher relapse rate with poor response to the conventional salvage therapy (Schnittger, *et al* 2002, Thiede, *et al* 2002). The outcome of patients with *FLT3*-ITD with resistance or relapse after initial therapy is particularly poor (Ravandi, *et al* 2010a). Therefore, there is a compelling need for novel therapeutic approaches for these patients with AML *FLT3*-ITD.

Recently, several FLT3 inhibitors (FLT3i) have been introduced to the clinic in multiple phase I -III clinical trials showing evidence of clinical efficacy (Pemmaraju, *et al* 2011). For instance, CEP-701, an orally available FLT3i, was used as a single agent for patients with AML *FLT3*-ITD and showed evidence of haematological response in some patients (Knapper, *et al* 2006, Smith, *et al* 2004). Midostaurin (PKC-412) also demonstrated evidence of clinical activity as a single agent (Fischer, *et al* 2010, Stone, *et al* 2005) and a phase III randomized clinical trial for frontline therapy in combination with chemotherapy is currently ongoing. Sorafenib, another potent FLT3i, showed evidence of clinical activity as a single agent in phase I studies (Metzelder, *et al* 2009, Zhang, *et al* 2008) and has also been combined with idarubicin and cytarabine for front line AML induction therapy (Ravandi, *et al* 2010b). AC-220 has been investigated in phase I and II clinical trials as a single agent in patients with refractory or relapsed AML. These studies reported not only a reduction of the blast cells but also complete remissions (CR) in some patients (Cortes, *et al* 2009, Zarrinkar, *et al* 2009).

The clinical efficacy of FLT3i, however, is mostly manifested by reduction of blast cells or by CR with incomplete haematological recovery (CRi), with the long-term benefit of such responses to patients still unclear. In addition, there is limited data to compare how patients treated with such approaches may fare compared to patients treated with conventional chemotherapy. To try to answer these questions we analysed and compared the outcome of patients treated with FLT3i at our institution to those treated with conventional chemotherapy to determine the long-term efficacy of therapy with FLT3i.

Methods

Patients

We conducted a retrospective study of 120 adult patients with AML having *FLT3*-ITD mutation who had relapsed after or exhibited disease refractory to front-line therapy, and thus received salvage therapy either under clinical trials or outside of clinical trials in our institution between August 1995 and June 2011. The cohort was divided into two groups according to the salvage therapy used: 1) a FLT3i (e.g., sorafenib, PKC-412, CEP-701, AC-220, KW-2449 and AP24534) either alone or in combination with chemotherapy

(“FLT3i group” hereafter), or 2) conventional cytotoxic chemotherapy (“conventional group” hereafter). All patients in the FLT3i group were treated under clinical trials while 13 out of 75 patients received salvage therapies outside the clinical trials in conventional group. Only patients receiving first salvage therapy with these agents were included in this study. Peripheral blood counts, blood chemistry, bone marrow aspirations and cytogenetic analyses were obtained before the start of salvage therapy. Patients who had received FLT3i as part of their frontline therapy were excluded from the analysis. Patients who carried *FLT3* D835 mutation in addition to *FLT3*-ITD were included, but those with only D835 without *FLT3*-ITD were excluded.

FLT3 mutational assay

Genomic DNA from bone marrow samples was isolated using the Autopure extractor (QIAGEN/Gentra, Valencia, CA). *FLT3*-ITD was determined by a semi-quantitative DNA-based polymerase chain reaction-capillary electrophoresis (PCR-CE) assay. The sensitivity of the assay for mutation detection was 1% as determined by dilution studies (Lin, *et al* 2006).

Response criteria and other term definition

Response to salvage therapy was classified according to the International Working Group (Cheson, *et al* 2003). Cytogenetic result was classified with favourable, intermediate and adverse prognosis according to the United Kingdom Medical Research Council (UK MRC) classification (Grimwade, *et al* 2001). Primary refractory identified patients never achieved CR with front-line therapy. CR duration was calculated from the time of CR until first relapse. Overall survival (OS) was calculated from the date of first salvage therapy until the date of last follow-up or death.

Statistical analysis

We reported frequencies, proportions, and measures of central tendency. We examined differences in the study groups regarding a variety of characteristics by using the Chi-square test or the Fisher's exact test for discrete variables. We used the Mann-Whitney *U* test for continuous variables. Overall survival was analysed using the Kaplan-Meier method and differences were compared using the log-rank test. Overall survival was calculated from the time of salvage therapy initiation until death or the last follow-up, where patients who were alive at the last follow up were censored. In order to evaluate the prognostic impact of FLT3i use in a multivariate setting, we performed a Cox proportional hazard regression analysis. We included variables with a p-value of ≤ 0.10 from the univariate model in addition to FLT3i variable into the multivariate model and obtained hazard ratio (HR) with 95% confidence interval (CI). A p-value (two-tailed) of <0.05 was considered statistically significant. We used IBM PASW Statistics 19 for Windows for statistical analyses (SPSS Inc., Chicago, Illinois).

Informed consent

This research protocol was approved by Institutional Review Board (IRB) of the University of Texas, MD Anderson Cancer Center and a written informed consent form was provided

to all patients in accordance with Declaration of Helsinki. Patients were also included in a retrospective chart review protocol approved by the same IRB.

Results

Patient characteristics

Of 120 patients with AML *FLT3*-ITD included in this analysis, 45 were included in the FLT3i group and 75 in the conventional group. The patient demographic and clinical characteristics at the time of first salvage therapy initiation are summarized in Table I. Median age, male to female ratio, and performance status were similar in both groups. White blood cell counts (WBC) and platelet counts before the initiation of first salvage therapy were not different between the two groups but patients in the FLT3i group had significantly lower haemoglobin levels. Two patients [4%] in the FLT3i group and 6 patients [8%] in the conventional group had *FLT3* D835 mutation in addition to *FLT3*-ITD ($P = 0.46$). The FLT3i group included fewer patients with diploid karyotype than the conventional group (24/45 [53%] vs. 61/75 [83%]; $P = 0.0012$) with a trend for more patients with adverse prognostic cytogenetic abnormalities (6/45 [13%] vs. 3/75 [4%]; $P = 0.062$).

In both groups, most patients had received a cytarabine (Ara-C)-based induction regimen (FLT3i group 38/45 [84%] vs. conventional group 71/75 [95%]; $P = 0.062$). Details of other induction regimens are described in Supplemental Table 1. Thirty-eight percent of patients in the FLT3i group and 35% in the conventional group had been refractory to induction therapy ($P = 0.73$). All other patients had relapsed with a median initial CR duration of 8.9 months (range, 0 to 39 months).

Salvage regimen

In the conventional group, the most common salvage regimen was a high dose Ara-C (HDAC)-based regimen (18/75 [24%]). Other salvage regimens used in the conventional group are detailed in Supplemental Table 2. The most common FLT3i used as salvage regimen were CEP-701 (18/45 [40%]) and sorafenib (18/45[40%]). Other patients in the FLT3i group received AC-220, KW-2449, AP24534 or PKC-412 (Supplemental Table 3). FLT3i was used as a single agent in 31/45 [69%] patients and was combined with cytotoxic chemotherapy in 14/45 [31%] patients. Detailed combination regimens are listed in Supplemental Table 3.

Thirteen patients in the conventional group received salvage therapies outside of the clinical trial. Pre-treatment clinical characteristics of these patients were not statistically different from those of patients treated under clinical trials (data not shown).

Stem Cell Transplant

Stem Cell transplant (SCT) was performed as part of the salvage therapy in 22/75 [29%] patients in the conventional group and in 11/45 [24%] patients in the FLT3i group ($P = 0.67$). Of those, 9/22 [40%] patients in the conventional group and 5/11 [45%] patients in the FLT3i group received SCT while they were in second CR ($P = 0.99$).

Response to salvage therapy

Response to salvage therapy is described in Table II. The rate of CR or **CRp** (CR without platelet recovery) was similar between the two groups (12/45 [27%] in the FLT3i group vs. 21/75 [28%] in the conventional group; $P = 0.99$). Further subgroup analysis was performed grouping the patients by CR duration, D835 mutation status and cytogenetic profile, but none of the subgroup analysis showed statistically significant difference in response (Table II). Also, we found no difference in the CR and CRp rate between patients who received FLT3i alone versus those who received FLT3i in combination with chemotherapy (Supplemental Table 4).

Overall survival

The median overall survival (OS) of all patients in the cohort was 4.8 months; it was 6.9 months (**95% CI; 3.6–10.3**) in the FLT3i group and 4.6 months (**95% CI; 3.5–5.8**) in the conventional group ($P = 0.17$) (Figure 1A). OS was not statistically different between the FLT3i group and conventional group in subgroup analysis of younger (age <60 years) and older patients (Supplemental Figure 1A and 1B). We then analysed survival by duration of first CR. In the patients whose first CR was shorter than 12 months or who were primary refractory to initial therapy ($N = 105$), the median OS was significantly better for those treated with FLT3i ($N = 43$) than for those in the conventional group ($N = 62$) (6.9 [**95% CI; 4.4–9.4**] months vs. 3.7 [**95% CI; 3.0–4.3**] months; $P < 0.01$) (Figure 1B).

Demographics and patients characteristics between FLT3i group and conventional group were not statistically different in this subgroup (primary refractory + first CR duration < 12 months) except that haemoglobin was significantly lower in conventional group, which was also manifested in the entire cohort (Supplemental Table 5). Further subgroup analysis was performed in the FLT3i group comparing patients who received FLT3i alone (monotherapy) or in combination with other chemotherapeutic agents (combination therapy). As shown in Fig 2A, the median OS was significantly better for those who received combination therapy as opposed to monotherapy (median OS 16.6 [**95% CI; 4.0–29.2**] months vs. 4.83 [**95% CI; 2.7–7.0**] months; $P = 0.017$); survival was also superior compared to patients in the conventional group (16.6 months [**95% CI; 4.0–29.2**] vs. 4.63 [**95% CI; 3.5–5.8**] months; $P = 0.023$).

In the entire cohort, univariate log-rank test showed that age younger than 60 years, Eastern Cooperative Oncology Group Performance Status (ECOG PS) lower than 2, bridging to SCT, and initial CR duration more than 12 months were associated with an improved survival (Supplemental Table 6). We fitted these significant parameters in the Multivariate Cox proportional hazard regression analysis with FLT3i use, which indicated that use of SCT, initial CR duration of more than 12 months and treatment with FLT3i were associated with better survival. After adjusting prognostic effect from other significant covariates, the use of FLT3i with the salvage therapy was found to favourably impact OS (HR, 0.58; 95% CI, 0.38–0.88). We found that an increment of one unit in the ECOG PS score at the time of first salvage was associated with an increase in the hazard of death of 74% (Table III). No significant interactions were found between the covariates included in the multivariate model.

Discussion

We present the results of a retrospective study of a large cohort of patients with AML *FLT3*-ITD where the long-term efficacy of FLT3i-containing salvage therapy was compared to conventional chemotherapy salvage. Our multivariate analysis showed that FLT3i-containing salvage therapy was associated with improved survival in a subset of AML patients with *FLT3*-ITD.

Patients with AML *FLT3*-ITD usually present with a high blast count, are more frequently identified among those with normal karyotype, and have a poor outcome manifested by early relapses and short survival (Frohling, *et al* 2002, Kiyoi, *et al* 1999, Kottaridis, *et al* 2001, Levis 2011, Levis, *et al* 2011, Rollig, *et al* 2011, Thiede, *et al* 2002). The outcome of patients with *FLT3*-ITD who have relapsed to prior therapy is very poor (Ravandi, *et al* 2010a). We recently reported an analysis of the clinical outcome of AML patients with *FLT3* molecular alteration compared to those with *FLT3* WT. In that study, the cohort with *FLT3* molecular alteration had worse response to first salvage therapy (CR 24% vs. 41% in *FLT3* WT) and worse median OS after first relapse (13 weeks vs. 37 weeks in *FLT3* WT) (Ravandi, *et al* 2010a). This is a similar population to the one we focused on for this analysis. Thirty six percent of all patients included in this analysis had been refractory to initial induction therapy and the median OS after the first salvage of our total cohort was 4.8 months, which reflects the high-risk feature of our study population.

A number of FLT3i have been developed and are currently being investigated clinically (Pemmaraju, *et al* 2011). These include agents such as sorafenib, CEP-701, quizartinib, midostaurin and others. Although reports of studies using these agents have reported evidence of clinical activity, most of this activity has been manifested by a frequently rapid and profound, albeit usually transient, decrease in the blast count. Most of the studies reported to date have been single-arm studies. For these reasons, despite the perceived benefit offered by these agents, a clear survival advantage has not been demonstrated for patients treated with FLT3i. To investigate the clinical impact of FLT3i-based therapy, we analysed the outcome of patients treated in our institution in a variety of phase I and II studies using FLT3i alone or in combination with chemotherapy. To better define the impact of therapy, we focused on a population of patients treated in a similar setting, i.e., receiving first salvage therapy after being refractory to first induction therapy or with relapse after first CR.

The subgroup analysis of our study suggests that patients who are refractory to induction therapy or who relapsed shortly after first CR (less than 12 months) had the best benefit from FLT3i salvage regimen. We carefully investigated whether there are any confounding factors in this subgroup that affect OS of FLT3i group. However, the demographics and haematological parameters were similar between FLT3i group and conventional group, suggesting OS benefit may indeed be indebted to the FLT3i use. Patients with short CR duration or primary refractory disease are usually considered to be refractory to standard chemotherapy and in fact, the results with standard chemotherapy as salvage therapy were poor (CR + CRp = 19%, median OS 3.7 months). The rate of CR + CRp was not significantly better with FLT3i salvage (26%) and yet patients treated with these agents had

improved survival. Interestingly, subgroup analysis of patients based on bridging to SCT showed that survival benefit from FLT3i use was only evident in patients who did not undergo SCT (data not shown). We can speculate on several explanations for the better outcome in the FLT3i-treated cohort. First, FLT3i therapies could have had less treatment-related mortality in a setting of salvage therapy. Patients who could not undergo SCT are perhaps less medically fit than patients who were bridged to SCT and FLT3i salvage may have been tolerated better in these patients. Second, the biology of AML *FLT3*-ITD could have been modified by FLT3i therapy. The allelic ratio of *FLT3*-ITD was almost ubiquitously decreased after FLT3i therapy in patients who had follow-up *FLT3*-ITD level determined after the salvage treatment. The allelic ratio of *FLT3*-ITD has significant prognostic implications (Gale, *et al* 2008, Schnittger, *et al* 2012, Thiede, *et al* 2002, Whitman, *et al* 2001) and salvage therapy by FLT3i may decrease *FLT3*-ITD burden in these patients thereby providing long-term benefit. Further, it is possible that achievement of CR is not the only surrogate marker for better OS in the setting of FLT3i-based therapy. In myelodysplastic syndromes, for instance, hypomethylating agents provide improved survival benefit for patients compared to cytotoxic chemotherapy even though both modalities equally provide haematological recovery. FLT3i therapy may mimic this scenario.. Our result also suggests that FLT3i may improve the outcome of AML *FLT3*-ITD patients when it is combined with other cytotoxic chemotherapy. Although sorafenib was combined with idarubicin and cytarabine in an induction regimen for AML patients, the experience with most of the FLT3i trial in both frontline and salvage setting is largely restricted to monotherapy (Ravandi, *et al* 2010b). Along the same line, *in vitro* studies have shown synergistic effect of FLT3i, such as CEP-701, with chemotherapeutic agents (Levis, *et al* 2004). Of interest, however, a randomized trial of chemotherapy with or without CEP-701 in the salvage setting did not show a favourable outcome (Levis, *et al* 2011). Such results could be considered a more direct evidence of a negative effect of FLT3i in contrast to our results from a retrospective analysis. However, it is possible that other FLT3i may be more effective clinically than CEP-701 and/or that other FLT3 inhibitor-based combination might be more effective than those used in the CEP-701 study. Our study has several limitations. Due to the retrospective design of the study, several biases were not avoidable, such as selection bias and data collection bias. Several different FLT3i were pooled in the analysis and, as mentioned above, these agents are not all equivalent in their potency and pharmacological properties. However, we designed this study to investigate the role of these drugs as a class of therapeutic agents, but clearly the effect of each individual drug needs to be investigated separately. We have performed several subgroup analyses, which always carry selection bias and decreasing number of the analysed patients. Additionally, our patient population is not homogeneous in terms of the timeframe in which they were treated. Patients in the FLT3i group were treated relatively more recently than those included in the conventional group, implying that the better survival in the FLT3i group may have been secondary to the advanced supportive care. Management and prevention of infectious complications associated with cytotoxic therapies are improving rapidly and those factors may have biased the OS benefit of FLT3i group. These biases can be minimized in prospective randomized controlled trials.

In conclusion, our study suggests that salvage therapy with FLT3i may improve the long term survival of AML *FLT3*-ITD patients whose disease is primary refractory or have short CR duration from initial chemotherapy. FLT3i might be particularly beneficial when combined with cytotoxic chemotherapy in the salvage setting. These results need to be confirmed in prospective studies in this and other settings (e.g., frontline therapy, later relapses) to confirm the possible clinical benefit of these agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Comparison of survival in (A) all patients and (B) patients whose complete remission duration was ≥ 12 months and who were refractory to induction therapy. Curves were

compared between FLT3 inhibitor (FLT3i) and conventional group. Small vertical lines indicate censored patients. Cum survival, cumulative survival.

Figure 2.

Kaplan-Meier analysis of survival of (A) patients who received FLT3 inhibitor (FLT3i) as monotherapy vs combination therapy and (B) patients who received FLT3i combination therapy vs conventional group. Small vertical lines indicate censored patients. Cum survival, cumulative survival.

Table 1

Demographic and clinical characteristics of 120 acute myeloid leukaemia patients with *FLT3*-ITD who were treated with either FLT3 inhibitors containing salvage regimen (FLT3i group) vs. conventional cytotoxic chemotherapies regimen (Conventional group).

	FLT3i group n = 45 (%)	Conventional group n = 75 (%)	P value
Age, years ($\pm$ SE)	55 $\pm$ 2.6	51 $\pm$ 1.8	0.21
Female (%)	51	55	0.71
ECOG PS			
0–1	41 (91)	62 (83)	0.2
2	4 (9)	13 (17)	0.2
Median Follow up (months)	16	18	0.73
WBC ($10^9/l$)	29 $\pm$ 4.8	40 $\pm$ 5.7	0.18
Hb (g/l)	91 $\pm$ 2.5	81 $\pm$ 1.8	0.001
Platelets ($10^9/l$)	99 $\pm$ 18	71 $\pm$ 6.5	0.086
Creatinine (μ mol/l)	79.6 $\pm$ 4.4	79.6 $\pm$ 6.2	0.73
Total bilirubin (μ mol/l)	8.6 $\pm$ 1.0	10.3 $\pm$ 0.7	0.32
LDH (iu/l)	1553 $\pm$ 234	1558 $\pm$ 133	0.99
With <i>FLT3</i> D835 mutation	2(4)	6(8)	0.46
Cytogenetics			
Diploid	24(53)	61(81)	0.001
Del 5 and Del 7	3(7)	1(1)	0.12
Trisomy 8	3(7)	1(1)	0.12
11q abnormality	0	1(1)	NA
inv(16)	0	2(3)	NA
Complex	3(7)	2(3)	0.29
Misc	12(27)	7(9)	0.01
Cytogenetics (MRC Classification)			
Favourable	0	2	NA
Intermediate	39	70	0.23
Adverse	6	3	0.06
Disease status			
Primary refractory	17(38)	26(35)	0.73
Relapsed	28(62)	49(65)	0.73
Induction regimen*			
Ara-C based	38(84)	71(95)	0.06
Other regimen	7(6)	4(5)	0.06

FLT3i, FLT3 inhibitors; ECOG PS, Eastern Cooperative Oncology Group Performance Status; WBC, white blood cell count; Hb, Haemoglobin concentration; LDH, Lactate dehydrogenase; Misc, miscellaneous chromosomal changes; MRC, UK Medical Research Council; NA, not applicable; Ara-C, cytarabine.

* Detailed in Supplemental Table 1.

Table II

Response* to salvage therapies according to disease characteristics.

	FLT3i group n/total (%)	Conventional group n/total (%)	P value
All patients	12/45 (27)	21/75 (28)	0.99
With D835 mutation	0/2 (0)	2/6 (33)	0.99
Without D835 mutation	12/43 (28)	19/69 (28)	0.99
Diploid cytogenetics	7/24 (29)	16/61 (31)	0.79
Favourable cytogenetics	0/0	2/2 (100)	NA
Intermediate cytogenetics	11/39 (28)	18/70 (26)	0.82
Adverse cytogenetics	1/6 (17)	1/3 (33)	0.99
Primary ref + CRD ≤ 12 months	11/43 (26)	12/62 (19)	0.48
CRD >12 months	1/2 (50)	9/13 (69)	0.99
FLT3i monotherapy	7/31 (23)	21/75 (28)	0.64
FLT3i combination	5/14 (36)	21/75 (28)	0.54

FLT3i, FLT3 inhibitors; NA, not applicable; Primary ref, primary refractory disease; CRD, complete remission duration.

* Response includes complete remission (CR) and CR without platelet recovery.

Table III

Results of multivariate Cox proportional hazard regression analysis for overall survival.

	P value	HR	95% Confidence Interval	
			Lower	Upper
Age (≤ 60 years vs. >60 years)	0.69	1.00	0.99	1.02
ECOG PS	0.036	1.33	1.02	1.73
Received stem cell transplantation	0.001	0.45	0.28	0.72
CRD (>12 months vs ref + ≤ 12 months)	0.001	0.35	0.18	0.66
FLT3i use	0.012	0.58	0.38	0.88

HR, hazard ratio; PS, Eastern Cooperative Oncology Group Performance Status; CRD, complete remission duration; ref, primary refractory; FLT3i, FLT3 inhibitors.